Pre –Ph.D. Registration Synopsis

STUDIES ON MICROBIAL META PROFILING AND BIOCHEMICAL ANALYSIS OF MILLET BASED HANDIA – A TRADITIONAL BREW OF JHARKHAND

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STATE OF ART

Our Country is rich in biodiversity, ethnic diversity with large variation of traditional knowledge and practice[1]. State of Jharkhand in India is a combination of topographically plateau with hills and plain areas. Ethnic diversity of Jharkhand is a huge tribal diversity (32 ST and 8 PVTG) having their own indigenous relying on the natural local flora and fauna[2]. The culture and practice of the ethnic people throughout the world and specially in Jharkhand has emerged through a continuous adaptation process between people and environment. The topographical features have the potential to be leveraged for improving dietary diversity, nutrition and health outcomes among the different tribal group. Tribal population of Jharkhand have a long history of preparing nutrient rich food and beverage from the traditional crops and local flora and fauna and this forms a broad bases of knowledge about the production ,preservation and utilization of the traditional food. So, tribes of Jharkhand have rich Traditional Ecological Knowledge (TEK).

Consumption of beverage both alcoholic and non-alcoholic is believed to have originated with origin of tribe way back in pre-Vedic period[3]. It has been found that the Socio-linguistic group throughout India have traditionally used consumption of fermented plant and grain for beverages production. The product obtain trough fermentation is till today ingrained into the existing Traditional Ecological Knowledge for fermenting and brewing different type of beverages (one of the oldest biotechnological techniques). It is very interesting to know there are various literature sources that almost every tribe has its indigenous alcoholic beverage and inclusive preparation as well as preservation techniques[3].With rapid development in the science and technology, there is a decline in the conservation of the invaluable traditional knowledge and practice and hence there is danger of completely losing such knowledge over a time if not documented properly. Therefore, preserving the indigenous traditional knowledge, the local ethnic practice, culture and health care practices that are of great benefit to the upcoming generation becomes the priority of the current generation[1]. The process and product control of the tribal drink can help to establish it a value based product and can link traditional indigenous knowledge to the main economy.

As already discussed, Jharkhand is a land of ethnic people rich in indigenous traditional practice like iron making by Asur tribes, handia making by almost all the tribe like Ho,

Munda, Santhal, Kharia. Handia is a traditional drink of Jharkhand. It is a traditionally prepared mild- alcoholic beverage mainly prepared from rice using local natural herbs in tablet form RANU by almost all the tribes of Jharkhand. Handia consumption has been a part of tribal history. Local oral history and finding add to the evidence to suggest that handia consumption has been a part of Jharkhand culture, rituals and traditions since time immemorial. Handia has played an important role in religions, typically seen as a gift to the divinities and closely associated with their workship[1]. The preparation process are varied, simple and authentic but are mostly region specific and tribal group specific. It is reported that the Ranu is prepared from different plants and its parts are locally available and those used as folk medicine for treatment of various disease[4]. This is known to protect from gastrointestinal aliments like dysentery, diarrhea, acidity and vomiting[4]. It exert a significant level of antioxidant activity. The making of handia is a housed practice and as reported by the indigenous group are different in different tribes and also the quality and nature of handia consumption and preparation has seasonal variation. It is said that the flavour of this drink changes with the serving hand. Each tribe has their own age old traditional rituals and believes and they follow the same while preparing the starter culture (RANU) and fermentation of handia[4]. The associations of different group of microbes and their relative concentration are essentials for the development of acceptable sensory quality of a traditionally fermented handia. The final fermented undistilled beverages are consumed directly and usually contain high amount of health beneficial components probiotics[1].

Probiotics as defined are live microorganism which when consumed in sufficient amount confer benefits on the host microflora[5]. Handia as reported by local ethnic people as well as literature confer benefit on the host in different way. The preparation of handia is an indigenous household practice of almost all tribes of Jharkhand utilizing traditional cooked rice (Gora, Karhani) supplemented with a starter culture RANU (balls made of ATAP RICE(arva chawal) and 32 herbs). The indigenous rice based drink is also a practice of other tribe in other region of India like West Bengal (tribe –Toto, drink – Eu)[3] ,Arunachal Pradesh (Adi Galo tribe , drink – Ennog)[6], Assam (Boro tribe ,drink – Jou bishi/ Jumai)[6] ,Odisha (Santhal tribe , drink- Rice beer /Handia)[7] etc.

There are several reports on the fermented beverages of different tribes but only a few pieces of literature are available on millet based handia. Personal observation and survey has revealed that there are few tribes who prepare handia from both rice variety and finger millet or Ragi. Handia preparation is an integral part of their culture and practice. It is

consumed by both male and female during festivals, marriages and religious ceremonies, etc[6]. Ragi based handia according to the tribes used as treatment for multiple diseases[8]. It is a natural relaxant that helps in relieving stress and anxiety[9]. According to Kharia people of Gumla district consumption of millet based handia prevents heart disease diarrhoea, bowel syndrome, removed weakness. They also reported during the corona period the consumption of Millet based handia helped them and people of their community didn't suffer from the Covid- infection. The freshly prepared millet based handia is given to the new mother for overcoming post-partum symptoms and also to infants for immune busting. Finger millets are expected to contain probiotic molecule[8]. The poor economic condition of the tribe led them to use the brew as a health drink or as a energy source. Rice based handia is one of the most consumed beverages in Jharkhand, but it is not safe for people with Celiac Disease. The demand of gluten free beverages is increasingly growing. Millet based handia is one of the good source of gluten free beverages.[9]

Work on the traditional beverage of Assam & West Bengal reports that the starter culture is a heterogenous mixture[3]of microbes which may be used as probiotics to increase the digestibility and other health benefits in the people consuming it. Literature also says that some bacteria present the starter culture are culturable and several other are non-culturable too[3].

Microbial Meta Profiling will be done by metagenomics profiling technique based on extensive sequencing of DNA have increase our knowledge on microbiome composition and activity[5]. The metagenomics profiling helps to define the fermentation processes because it genetically characterized each microorganism present in a starter culture and may ensure quality control and reproduciblity of these traditional beverages[5]. It can also help in the scaling process of these beverages[10]. The development of these technique is also contributing to many different aspects of probiotic research[5].

Phytochemical and Biochemical profiling of earlier work suggest that rice and millet based indigenous beverage prepared by the ethnic people throughout the world having nutritional as well as medicinal value even antioxidant along with some mild-hallucinations effect[11]. Rice based handia prepared by every tribe and millet based handia prepared specifically by few tribe (munda) though prepared by each and every tribe of Jharkhand specially representing one of the oldest biotechnological process is still having no scientific standardization and quality control as a probiotics. No scientific work has ever been undertaken for the microbial

population evaluation or biochemical and microbial meta-profiling in millet based handia prepared in Jharkhand.

Many work on the documentation of the herbs and plants used in RANU preparation has been undertaken[4] but till date no work on the making and promotion of this drink as a probiotics has ever been done. The present study tries to undertake the identified gaps in the field of making ,quality and promotion of the drink as probiotics.

PROBLEM TO BE INVESTIGATED

Microbial meta-profiling and biochemical analysis of millet based traditional tribal drink (handia) for its promotion as probiotic.

OBJECTIVE OF INVESTIGATION AND MOTIVATION

The purposed work **"STUDIES ON MICROBIAL META-PROFILING AND BIOCHEMICAL ANALYSIS OF MILLET BASED HANDIA – A TRADITIONAL BREW OF JHARKHAND"** has been undertaken with following broad objectives **:-**

- Survey and Documentation of indigenous preparation and maintenance of handia.
- Biochemical analysis of millet based handia.
- Microbial Meta-profiling of starter culture of handia.

MOTIVATION BEHIND THE WORK

Handia though now a days treated as alcoholic, a toxicating beverage in Jharkhand, in its original form of preparation and consumption were medicinal and nutritive drink enrich with some nutritional and medicinal value. This traditional beverage is facing problem of natural ingredients. The changing climate and in absence of standardized control scientific protocol. The purity and secret value of this drink is facing challenges and the original form of preparation and consumption is at the verge of extinction. So, the main motivation behind the work is conservation of the traditional handia and all culture around it, economic revival of the heritage cultural practice by establishing the phytochemical and micobes for standard scientific protocol development, market linkage of the Vedic old biotechnological process for its promotion as a probiotic drink.

HYPOTHESIS

Documentation and preservation of intangible technologies for traditional beverage preparation in Jharkhand. The economic revival of heritage cultural practices by establishing

biochemical and microbes of millet based beverage for its marketing and conservation of the pre - Vedic old traditional biotechnological indigenous technique and its promotion as probiotic for the present and future generation.

RESEARCH DESIGN & METHODOLOGY

Methodology will be in three parts :-

1. **Survey and documentation**
    a) Literature survey
    b) Field survey
        - Study area - **some area of Ranchi and Khunti district (JHARKHAND)**
        - Survey and documentation of ingredients of starter culture (RANU) and handia.
        - Survey and documentation of process of RANU preparation and handia preparation.
        - Preparation of People Biodiversity Register.
        - For collecting data from field audio visible tools i.e mobile, camera will be used.

There are 2 types of handia mainly prepared in Jharkhand –

a) Rice based handia
b) Millet based handia

Present laboratory work based on Millet based handia sample collected from tribal family of either Ranchi or Khunti district.

2. Laboratory based work:-

i. Sample collection and preservation

a) Collection of raw materials
b) Collection of handia sample and starter culture and were preserve in laboratory for analysis.

ii. Biochemical analysis of sample

- Alcohol content
- pH determination
- Pesticides analysis
- Heavy metal analysis

iii. Qualitative screening of sample

Qualitative screening of sample including carbohydrate, alkaloids, phenol, flavonoids, tannin, ascorbate, terpenoids, steroids, glycoside will be done by standardized protocol.

iv. In vitro antioxidant assay

In vitro antioxidant activity will be perform to determine the free radical scavenging activity of the samples.

v. Quantification of total phenol and flavonoid content

vi. Gas chromatography-mass spectrometry (GC-MS) analysis

The bio- active compounds of the sample will be determined by the GC-MS analysis.

vii. Analysis of nutritional content

- Estimation of carbohydrate
- Estimation of protein

viii. Microbial Meta-profiling of starter culture

ix. Bio statistical analysis

MATERIALS TO BE USED IN THE COLLECTION OF DATA

Sample required -

i. Millet

ii. Starter culture (RANU)

iii. Millet based handia

Apparatus required-

Test tubes, Watch glass, Test tube holder, Oven, Test tube stand ,Beaker, Conical flask, Sample container, Funnel, Measuring cylinder, Cubit, Falcon tubes, filter tip, Aliquot, Burner, Burner Chamber, Cotton plug, Spatula etc.

Various chemicals required

Ethanol, Methanol, Sucrose, Iodine, Potassium, Ferric chloride, Magnesium, Hcl, Sodium hydroxide, Chloroform, Conc. Sulfuric acid, Felhings reagent etc.

Machine required-

Refrigerator, Weighing machine, Water bath, Centrifuge, Autoclave, Hot air oven, Vortex machine, Laminar airflow chamber, Incubator etc.

Place of Work

Work is mostly laboratory based and thus will be done in University Department of Botany (RANCHI UNIVERSITY), Metagenomics Analysis will be done in Kolkata and GC-MS Analysis will be done in BIT Mesra, Ranchi**.**

SIGNIFICANCE OF THE STUDY

The proposed study is of for reaching significance:-

1. It will help to understand the features and medicinal uses of traditional drink of tribal people of Jharkhand and also present status of its microbial meta-profiling study.
2. Qualitative and Quantitative analysis of handia will be help in study of microbial association free radical scavenging activity**.**

IT'S RELEVANCE TO PRESENT DAY PROBLEMS AND NEED OF THE SOCIETY

1. Millet based traditional tribal drink prepared by tribal people is a traditional beverage prepared indigenously in a very scientific and précised old traditional culture but in due course of time due to urbanization and intermingling of western culture these beverages are now a days treated and consumed as alcoholic beverages.
2. The beverage which was in its original form nutritive and medicinal is now being replaced with the local daru. So, there is an urgent need to give protection to this tradition old culture and practice in Jharkhand were its consumption is a daily practice of almost all the tribal group.

3. The millet based traditional beverages are facing problem of natural ingredient. Climate change is also affected the quality of this drink.

4. There is no any documentation or standardization protocol for the preparation of this drink in absence of any standardized control scientific protocol, the purity and separate value of this drink is facing challenges and the originality of this drink is at the verge of extinction.

5. People of the both tribal and non-tribal community for easy money making are trying to sell this drink as an alcohol with high alcoholic concentration adding synthetic chemicals like urea, rat poison, tobacco which is causing many health problems among the tribal people of almost all age groups.

6. If immediate conservation measures are not taken then this pre –Vedic old traditional practice will lose its ethnicity and will become only a substitute of local daru.

ECONOMIC REVIVAL

1. The purposed work is the need of the tribal society for this will help towards documentation and preservation of intangible technologies of the tribal people and also helpful to reveal some new scope for value addition in this traditionally prepared indigenous drink.

2. The economic revival of the cultural practice by stabilizing biochemical and microbes for its market linkage is also needed to conserve this old biotechnological process.

3. The drink also needs its promotion as probiotic drink of present and future.

CONTRIBUTION TO THE EXISTING KNOWLEDGE

1. Documentation of the ingredients and process of preparation of this indigenous drink prepared by tribal people will provide a scientific data in the traditional knowledge database.

2. The documentation of the process of handia preparation will help in bring out the presently adopted method of this drink and help to identify the present gap in the traditional method of preparation and consumption and present method of preparation and consumption which will help to conserve the ethnicity of this drink.

3. Biochemical profiling of this drink will help to identify its medicinal and other beneficial property among the consuming tribe.

4. Microbial Meta-profiling will help in analysing and reporting the beneficial microbes present in this drink.

FUTURE WORK

1. This study for the first time emphasizes the indigenous practice of beverages preparation by the tribal people of Jharkhand.
2. The work will help to understand the features and medicinal uses of the beverage in Jharkhand and also help the researcher to undergo its genomic study.
3. A future work on the value addition of this beverage by research and method development, microorganism selection, raw material improvement may be done.
4. Standardization and value addition to this drink in future may led to their industrialization .Which will be beneficial for the economic sustainability of tribal people and other such tribes preparing indigenous drink.
5. The microbiological propititate and toxicity testing will help in the promotion and commercialization of this drink as future probiotics.

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